HYBRIDIZATION AND GENETIC RECOMBINATION OF CIRSIUM CALIFORNICUM AND C. OCCIDENTALE (ASTERACEAE: CARDUCEAE)

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ABSTRACT

Sympatric populations of *Cirsium californicum* (series *Neomexicana*) and *Cirsium occidentale* (series *Occidentalia*) were studied. The populations occurred as a set of colonies along Happy Canyon Road, Santa Barbara County, California. Morphological data, pollen fertilities, and controlled crosses all support the conclusion that the two taxonomic species studied are one biological species at the Happy Canyon site, that no sterility barriers exist to prevent gene recombination, and that hybrid and recombinant forms compose almost the entire Happy Canyon *Cirsium* population. However, microgeographic differentiation of recombinant morphological types was observed to be correlated to ecological habitats. Protein electrophoretic data support the conclusion that habitat-correlated electrophoretic and morphological phenotypes have a genetic basis rather than having been environmentally induced. The data suggest that the two species have hybridized in the studied population and that differentiation may be occurring along new lines.

The genus *Cirsium* is in the family Asteraceae, tribe Carduceae (Thistle). Taxonomically, there are between 200 and 250 described species in the genus, of which 34 occur in Washington, Oregon, and California (Howell 1968). Approximately 30 of the West Coast species occur in California (Munz and Keck 1959). The classification of the California species of *Cirsium* has been changed several times in this century (Jepson 1925, Munz and Keck 1959, Howell 1968, Munz 1974). All of these authors have suggested that the taxon *Cirsium* is evolutionarily complex and in need of further study before its evolutionary dynamics can be completely understood.

Taxonomic division of *Cirsium* into species is made difficult in part by the presence of morphologically intermediate individuals. Hybridization of Pacific Coast species has been suggested between approximately 23 species pairs involving 19 species (Howell 1968). Hybridization occurs not only between closely related species, but also between some morphologically dissimilar species (*C. fontinale* and *C. quercetorum,* and *C. brevifolium* and *C. utahense*). It appears that the genus is taxonomically complex and that the difficulty of species delimitation may result from rapid, present-day evolution.

Despite the several revisions of the genus, *C. californicum* and *C. occidentale* have always been considered distinct; they have always been placed in separate series and have never been reported to hy-

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bridize. Acceptance of the current ranking of *C. californicum* and *C. occidentale* is based on the apparent historical continuity of classifications and agreement of taxonomists (Jepson 1925, Munz and Keck 1959, Howell 1968, Munz 1974).

This study presents data on the interactions of *C. californicum* and *C. occidentale* occurring sympatrically in a single well-defined geographic population. Two questions are addressed. First, what degree of biological distinctness has been reached between the species *C. californicum* and *C. occidentale* in the study area? Second, what, if any, geographically related genetic differentiation exists in the studied population? Answers to these questions may lead to a greater understanding of the processes of evolution in nature and ultimately of the phylogeny of *Cirsium*.

Materials and Methods

Species description. C. californicum branches from the base upwards. The plants have a strong taproot. Basal leaves develop in a rosette and can be up to 3 dm long and 1 dm wide. They are oblanceolate, deeply lobed, and have slender spines. Caulescent leaves are shorter, and have reduced lobes and spininess distally. Leaf blades are glabrescent and green above, and white arachnoid-wooly below. The capitula of *C. californicum* usually occur solitarily on the ends of long slender peduncles. The heads are up to 6 cm in diameter, and up to 4.5 cm long. The involucre is bowl shaped, making the capitulum hemispheric. Phyllaries are spine tipped and spreading above. Flowers are tubular and white, sometimes pink (Table 1).

C. occidentale is an herbaceous, taprooted plant up to 3 m tall. Basal leaves form a rosette up to 8 dm in diameter from which a single thick stem arises. Branching occurs only on the upper stem and forms the inflorescence. Leaves are purple to reddish with light cottony pubescence, deeply lobed, and very spiny. Deep lobing and spininess occur from the leaf base to the distal tip. The branching pattern forms a panicle. Flowers in the heads are compressed into a vertical cylinder by the phyllaries. Phyllaries are constricted to form a narrow neck, are spine tipped, and have cottony webbing. Flowers are all tubular, perfect, and red (Table 1).

The population. The population studied was composed of *C. californicum* and *C. occidentale* individuals in an essentially linear array of 42 disjunct colonies along Happy Canyon Road to the summit of Figueroa Mountain, Santa Barbara County, California. In this respect it resembled the linear stepping stone model of population structure (Kimura 1953). The population was dispersed over approximately 19 km and elevation ranged from approximately 600 to 1100 m. Four habitat types were traversed by the Happy Canyon roadway with its associated *Cirsium* population: chaparral, valley grassland, southern

TABLE 1. MEAN AND STANDARD ERROR FOR EACH OF THE 35 MORPHOLOGICAL CHARACTERS. Key: [1] Includes reference specimens and individuals fitting species description which were used in the experimental crosses. [2] n = 4, n = 1, n = 34, and n = 105, respectively (not all specimens had mature seeds). * Denotes characters whose means are significantly different in *C. occidentale* and *C. californicum* (±2 S.E. do not overlap).

Character in cm except ±0/1 and #	*Cirsium occidentale*[1]		*Cirsium californicum*[1]		Principal coordinate axis III			
					+ cluster		− cluster	
	$\bar{x}$ n = 16	S.E.	$\bar{x}$ n = 17	S.E.	$\bar{x}$ n = 66	S.E.	$\bar{x}$ n = 206	S.E.
Stem leaf:								
Length	12.86	0.74	12.05	1.00	10.07	0.68	10.72	0.41
Width*	1.34	0.14	0.86	0.08	1.00	0.09	0.80	0.03
No. lobes	15.88	0.52	13.24	1.15	13.64	0.50	15.19	0.26
Spine length*	0.49	0.04	0.28	0.02	0.42	0.02	0.40	0.01
Lobe depth	0.95	0.12	0.89	0.14	0.67	0.05	0.69	0.03
Color ±*	0.94	0.06	0.00	0.00	0.49	0.06	0.30	0.03
Pubescence ±*	0.94	0.06	0.18	0.10	0.05	0.03	0.98	0.01
Peduncle leaf:								
Length*	2.51	0.30	1.38	0.16	1.54	0.08	1.87	0.05
Width*	0.36	0.06	0.14	0.02	0.17	0.01	0.18	0.01
No. lobes*	8.00	1.03	3.65	0.43	6.38	0.44	5.48	0.20
Spine length*	0.37	0.03	0.23	0.02	0.32	0.02	0.29	0.01
Lobe depth	0.16	0.02	0.09	0.02	0.10	0.01	0.12	0.01
Color ±*	0.88	0.09	0.00	0.00	0.47	0.06	0.31	0.03
Pubescence ±*	0.94	0.06	0.18	0.10	0.39	0.06	0.88	0.03
Peduncle:								
Length	16.15	2.85	23.94	4.13	17.85	1.34	17.68	0.68
Diameter*	0.33	0.02	0.21	0.02	0.22	0.01	0.23	0.01

TABLE 1. CONTINUED.

Character in cm except ±0/1 and #	*Cirsium occidentale*[1]		*Cirsium californicum*[1]		Principal coordinate axis III			
					+ cluster		− cluster	
	$\bar{x}$ n = 16	S.E.	$\bar{x}$ n = 17	S.E.	$\bar{x}$ n = 66	S.E.	$\bar{x}$ n = 206	S.E.
Capitula:								
Bract length*	1.72	0.23	1.00	0.10	1.29	0.05	1.49	0.03
Bract width	0.28	0.02	0.24	0.02	0.29	0.01	0.31	0.01
Total height*	4.38	0.21	3.31	0.10	3.68	0.09	3.60	0.04
Bract pubescence ±*	0.81	0.10	0.00	0.00	0.41	0.06	0.20	0.03
Involucre:								
Width*	2.61	0.16	1.88	0.13	2.10	0.06	2.30	0.04
Height*	2.96	0.20	1.94	0.10	2.30	0.07	2.19	0.04
Corolla:								
Tube length*	2.06	0.13	1.53	0.09	1.65	0.04	1.72	0.02
Throat width	0.11	0.01	0.11	0.01	0.10	0.00	0.10	0.00
Lobe length*	1.01	0.10	0.65	0.02	0.79	0.03	0.74	0.01
Lobe shape ±*	0.81	0.10	0.00	0.00	0.38	0.06	0.27	0.03
Anther:								
Length	0.99	0.06	0.86	0.05	0.92	0.03	0.95	0.04
Tail length	0.11	0.01	0.07	0.01	0.11	0.01	0.11	0.01
Length past corolla	0.87	0.09	0.59	0.06	0.79	0.04	0.72	0.02
Stem diameter*	0.95	0.07	0.62	0.05	0.86	0.04	0.79	0.03
Pappus length*	2.18	0.09	1.55	0.06	1.88	0.06	1.84	0.02
Seed length[2]	0.61	0.01	0.50	—	0.64	0.01	0.65	0.01
Seed width[2]	0.29	0.01	0.20	—	0.26	0.01	0.27	0.00
Stem color ±*	0.81	0.10	0.00	0.00	0.49	0.06	0.32	0.03
Stigma length	0.56	0.06	0.48	0.04	0.46	0.02	0.48	0.01

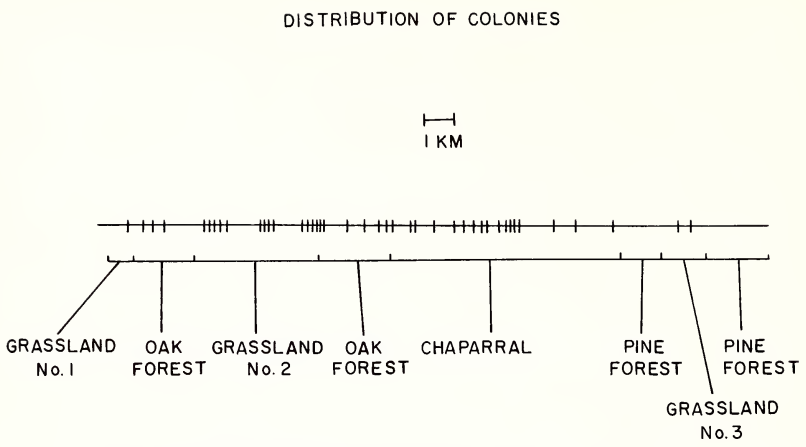

FIG. 1. Map of colony and habitat distribution for Happy Canyon *Cirsium* population.

oak woodland, and yellow pine forest (Munz and Keck 1959). Colonies of the Happy Canyon *Cirsium* population were distributed such that 41% were in the chaparral region; 38% in the grassland areas; 21% in the oak woodland, and none in the pine forest (Fig. 1). A survey of the terrain away from the road revealed that Happy Canyon *Cirsium* occurred only in roadside colonies.

Elevation increased most rapidly in the chaparral area (400 m) and least in the nearly level grassland regions. Three separate grassland areas existed, isolated from one another by large areas of other habitat types. Grasslands No. 1 and No. 2 were spatially close and at approximately the same elevation, whereas grassland No. 3 was isolated from both grasslands No. 1 and No. 2 by large areas of other habitat types and was at a significantly higher elevation.

Even though *Cirsium* colonies do not extend into the native, undisturbed vegetation in the study region, habitat type is an indicator of and modifies, the overall environment. That is, the species composing the surrounding community are a result of the environment of a region, and may be a better measure of the environment than can be obtained through direct measurements, because even if many environmental parameters are measured at many locations, one still does not know the relative weighting of the factors (Unger 1836, Schimper 1898, Warming 1909, Drude 1913, Odum 1959, Ovington 1962, Daubenmire 1968, Krajina 1969, Mueller-Dombois and Ellenberg 1974). Thus, it is as a reflection of the environment, rather than extensive interaction of *Cirsium* with species in the community, that I refer to habitat type.

Colonies of *Cirsium* consisted of discrete patches of individuals oc-

curring at densities between 1 and 4 plants per m^2. When *Cirsium* occurred on both sides of the road at a location they were considered a single colony. Approximately 200 flowering individuals occurred in each colony, except the grassland colonies, which ranged between 500 and 1000 flowering individuals. The only colonies with fewer individuals were the three chaparral colonies adjacent to the pine forest habitat, and the isolated grassland colony No. 1 in Fig. 1. The study was carried out from 1976 to 1979, and collections were made in 1977.

Morphological data and analysis. A random sample of each colony was obtained by collecting plants along a line transect. Two hundred thirty-two plants from the Happy Canyon population (an average of 5.5 plants per colony) were chosen from the collections, using a random numbers table, for numerical taxonomic analysis. In addition, 15 plants used in crosses and chosen for their similarity to *C. californicum, C. occidentale,* or for their intermediacy, were included in the morphological analysis. Finally, included were 12 herbarium specimens of *C. californicum,* 11 of *C. occidentale,* 2 of apparent hybrid identity (Santa Cruz Island and Tule River), a specimen of *C. vulgare,* and a specimen of *C. tioganum,* all identified previously by other collectors. Specimens were all from the University of California at Santa Barbara Herbarium (UCSB 7229–7235, 7239–7244, 6454, 6456, 6459, 8440, 15330, 18245, 22061, 22291, 24011, 24291, 24320, 28865, 31527, 32531). The *C. californicum* and *C. occidentale* herbarium specimens were used as references for species phenotypes. By using specimens identified and reviewed by other systematists, personal biases were eliminated in establishing references.

Thirty-five morphological characters were used in the numerical taxonomic analysis (Table 1): 28 metric and 7 plus-minus (+ pubescence = dense; + color = red; + corolla lobe shape = spatulate). Characters were chosen that showed variability in the population and that were obtainable from herbarium specimens as well as living material. Characters were measured on plants of similar age, and on organs of comparable development.

All characters were ranged between values of 0 and 1 before Q matrix numerical analyses, so that they all had equal weight (Sneath and Sokal 1973). Morphological data analysis was performed by the distance diagram technique (Wells 1979), cluster analysis (NTSYS Program), and principal coordinate analysis (Gower 1966), all of which operate on Q type matrices. Principal component analysis (SPSS Program) and means and their standard errors were obtained from R type matrices. Analyses were performed on an IBM 360/75 computer. The generalized n row by m column hypergeometric version of the general exact test (Wells and King 1980) and the chi-square test were used to test for a relationship between habitat type and morphological form, as defined by the principal coordinate analysis. The spatial (11.5 km)

and elevational (400 m) isolation between *Cirsium* of grassland No. 3 and No. 1 & 2 allowed testing of the hypothesis that habitat correlated selection had occurred, rather than formation of a cline or selection by an environmental factor not related to habitat.

The alternative hypotheses were considered by testing for differences between the *Cirsium* of grassland areas 1 & 2 and 3, between the *Cirsium* of grassland 1 & 2 and chaparral, and between grassland 3 and chaparral *Cirsium* using the chi-square and general exact test statistics. If a cline existed or selection by a factor actually uncorrelated to habitat occurred, then *Cirsium* in grassland areas 1 & 2 and 3 should be different because they are isolated and at different elevations. However, grassland *Cirsium* from areas 1 & 2 and 3 should not differ, but each should be different from chaparral *Cirsium,* if habitat determining factors are causing differentiation. Finally, silhouettes of stem leaves, inflorescences, and peduncle leaves provide a graphic display of the variation within the Happy Canyon population.

Sterility barriers. The presence of sterility barriers to gene recombination was tested by observing pollen fertility, and by ovule development in controlled crosses, as well as by morphological analysis. Ownby et al. (1975) have studied *C. californicum* and *C. occidentale* cytologically and demonstrated that both have $2n = 30$ chromosomes throughout their range. Percent pollen fertility was tested on the 274 plants used for morphological analysis. A flower was removed from each of the dried specimens, 200 grains sampled and stained with lactophenol blue to determine percent fertility (Radford et al. 1975). Fertility barriers were also tested by crosses between species, within species but between individuals, between species types and hybrid types, and by bagging. An analysis of variance on the arcsine $\sqrt{\text{frequency}}$ of developed ovules was used to determine significance of differences. Finally, 1160 capitula, each from a separate plant (a sample independent of those used for morphological analysis), were dissected and the percentages of developed ovules recorded.

Protein electrophoretic data and analysis. Seeds removed from the inflorescences collected to determine the field ovule development of heads were germinated and grown to small rosettes. Leaves of the rosettes were removed and used (while still fresh) for electrophoresis in the isozyme analysis. Slab acrylamide gel electrophoresis was used to separate isozymes following the techniques described by Wells and Wells (1980). The enzyme loci studied include leucine aminopeptidase (LAP), alkaline phosphatase, acid phosphatase, malate dehydrogenase, lactate dehydrogenase, and "general protein." Isozyme stain formulas are those of Shaw and Prasad (1970) and Johnson (1975). Results were grouped according to habitat type. The hypergeometric version of the general exact test (G.E.T.) and the chi-square test were used to determine whether significant differences occurred in genotype fre-

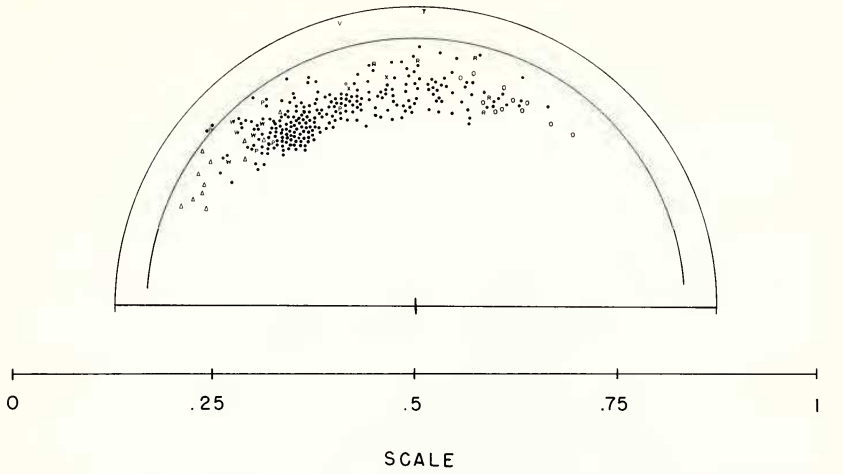

FIG. 2. Distance diagram of Happy Canyon and herbarium reference individuals. *Cirsium occidentale* and *C. californicum* reference specimens fall into two distant clusters according to species. *Cirsium vulgare* and *C. tioganum* are depicted as distinct species by their position. Happy Canyon individuals exist as a continuum of points from the *C. occidentale* to the *C. californicum* herbarium specimens as would be expected if hybridization and backcrossing are occurring. △ = *C. californicum,* ○ = *C. occidentale,* ● = Happy Canyon *Cirsium* individuals, V = *C. vulgare,* T = *C. tioganum,* and X = *Cirsium* herbarium specimens of questioned species identity. Individuals used in crossing experiments: R = red flowered plants resembling *C. occidentale,* W = white flowered plants resembling *C. californicum,* and P = pink flowered plants intermediate in floral characteristics.

quencies between colonies grouped by habitat type. Both the general exact and chi-square tests were also used to test whether habitat related selection was actually occurring by comparing grasslands 1 & 2 and 3, and to chaparral *Cirsium,* as was done with the morphological data. Genotype frequencies rather than allele frequencies were used because random mating could not be assumed, and because they are more conservative (sample size ½) than inferred allele frequencies (Spiess 1977).

RESULTS AND DISCUSSION

One biological species or two? Morphological variation in the population, pollen fertility within the population, and seed set in experimental crosses were examined to determine whether *C. californicum* and *C. occidentale* are true biological species in the Happy Canyon population. Morphological data are displayed in a distance diagram (Wells 1979) in Fig. 2; a phenogram from a cluster analysis is given as Fig. 3; scatter diagrams of the first three axes of a principal coordinate analysis (Gower 1966) in Figs. 4, 5; as means and their standard

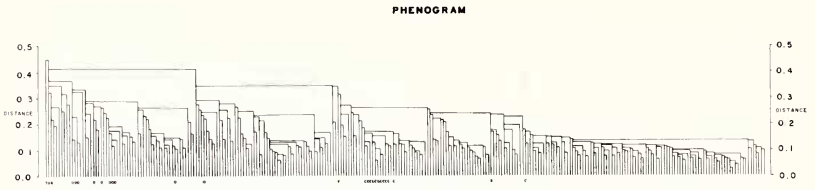

FIG. 3. Phenogram from unweighted pair group method using arithmetic average linkage cluster analysis. The lack of large differences in the cluster levels indicates a gradation of morphological forms, as would be expected through hybridization and backcrossing. Herbarium specimens: O = *C. occidentale,* C = *C. californicum,* T = *C. tioganum,* V = *C. vulgare,* and X = *Cirsium* herbarium specimens of questioned species identity.

errors in Table 1; and as stem leaf, inflorescence, and peduncle leaf silhouettes in Figs. 6, 7, 8.

Distance diagram. The herbarium specimens identified as *C. californicum* and as *C. occidentale* fell into two distinct clusters along species lines (Fig. 2). *Cirsium vulgare* and *C. tioganum* herbarium specimens fell outside the inner semicircle and close to the outer semicircle centrally in the diagram, as should distinct species. Also note that two herbarium specimens of hybrid identity (Santa Cruz Island; and Tule River, Tulare County, California) fell at an intermediate "hybrid" position on the distance diagram.

The study population was a continuum of forms, ranging from *C. californicum* to *C. occidentale* within both circles (Fig. 2). Of 247 plants of the Happy Canyon sample, only two fell outside the inner semicircle of the distance diagram, as might be expected if backcrosses frequently occur and neither parental line contains all alleles of some genes for extreme character values (Grant 1964). All evidence from the distance diagram is consistent with the hypotheses that only one biological species was being studied in Happy Canyon, that no sterility barriers existed, that hybridization and backcrossing are common, and that Happy Canyon *Cirsium* may be treated as one genetic population.

The distance diagram also includes the individuals used in crosses that will be analyzed later. Some *C. occidentale* individuals used in crosses appear to be backcrosses, but as Fig. 2 depicts, examples of *C. californicum* and *C. occidentale* as defined by the reference individuals were difficult to find.

Cluster analysis. One sees no distinct clusters, but rather a gradation of cluster levels in Fig. 3. If there had been a barrier to initial hybridization and subsequent backcrossing, two distinct clusters should have been produced by the cluster analysis, corresponding to *C. californicum* and *C. occidentale,* respectively. The cluster analysis, like the distance diagram, supports the interpretation that a single genetic

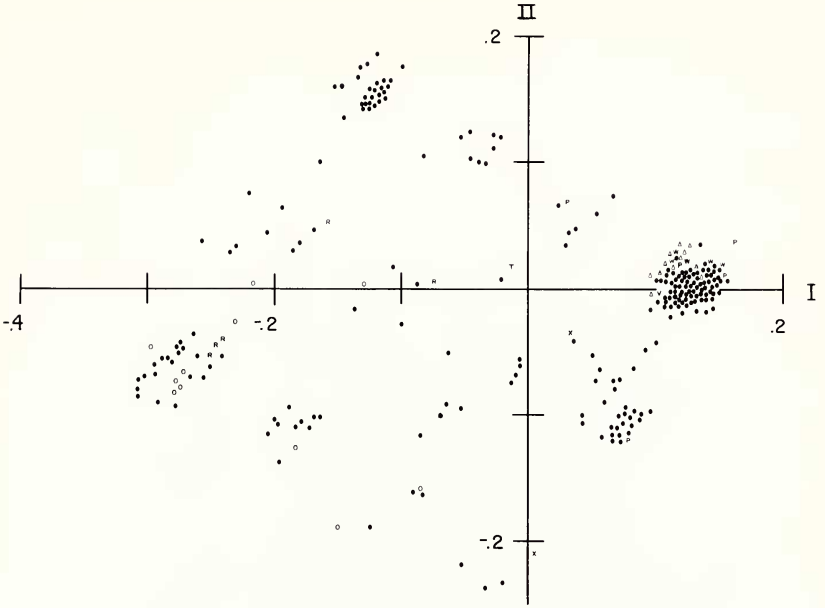

FIG. 4. Principal coordinate analysis axes I and II. No distinct clusters occur as expected when hybridization and backcrossing occurs. Herbarium specimens: O = *C. occidentale*, △ = *C. californicum*, T = *C. tioganum*, V = *C. vulgare*, and X = *Cirsium* herbarium specimens of questioned species identity. Individuals used in crosses: R = *C. occidentale*, W = *C. californicum*, and P = intermediate. ● = Happy Canyon *Cirsium* individuals.

population was being studied with no sterility barriers between morphological types.

Principal coordinate analysis. Next, individual morphological variation was studied through principal coordinate analysis. The first three axes account for 64% of the total variance; the first 13, for 93% of the total variance. Depicted as Fig. 4 are axes I and II, and as Fig. 5 are axes I and III. If barriers exist to gene exchange and recombination, the principal coordinate analysis should depict just two isolated clusters of points: one containing all of the *C. californicum* reference individuals, and the other all of the *C. occidentale* reference individuals. Axes I and II reveal no isolated cluster of points; a continuum exists between *C. occidentale* grouped reference points and those of *C. californicum*. Figure 4, therefore, gives evidence that only one genetic population exists.

Figure 5, which displays axes I and III of the principal coordinate analysis has new information, but still supports the conclusion that one biological species was being studied and that there were no bar-

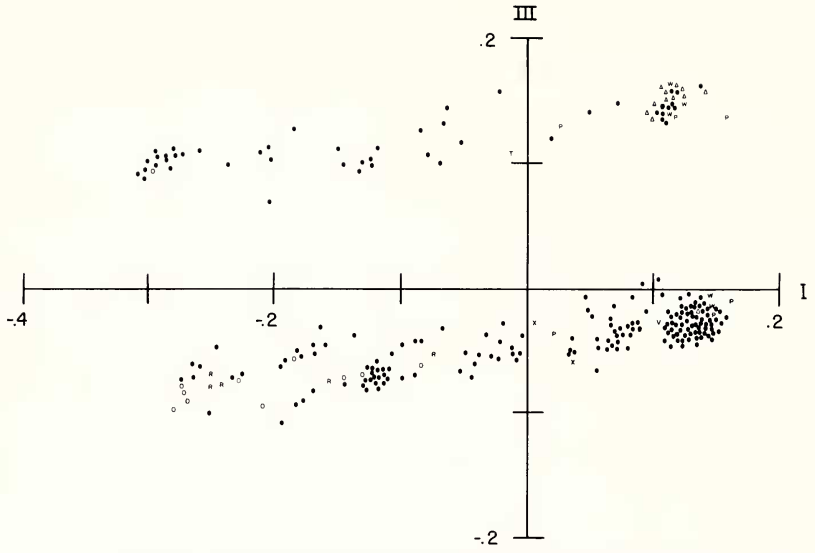

FIG. 5. Principal coordinate analysis axes I and III. Two distinct clusters occurred corresponding to the positive and negative poles of axis III. However, reference individuals of both *C. californicum* and *C. occidentale* occurred in both clusters, and within a cluster a continuum of forms exists between the species types. Therefore, some factor (e.g., environmental selection) other than species has caused differentiation of two recombinant forms. Herbarium specimens: O = *C. occidentale,* △ = *C. californicum,* T = *C. tioganum,* V = *C. vulgare,* and X = *Cirsium* herbarium specimens of questioned species identity. Individuals used in crosses: R = *C. occidentale,* W = *C. californicum,* and P = intermediate, ● = Happy Canyon *Cirsium* individuals.

riers to hybridization. Clearly, two clusters occur in Fig. 5, one on the positive side of axis III and the other on the negative. However, reference individuals of both species, and of both species types used for crosses, occur in both clusters. Also, in both the positive and negative clusters there is a continuum of forms spanning the space between the reference specimens of both species. Thus, the morphological evidence still supports the theory that there is but one biological species being studied, and that recombinant forms constitute the majority of the individuals of the studied population. However, there appears to be grouping of recombinant phenotypes into two clusters by some force (genetic or developmental response). Principal coordinate analysis of axes II and III (available from the author) gave results similar to axes I and III. Axis three still caused separation into two clusters, and each cluster still had both reference species included.

Principal component analysis. A principal component analysis (R matrix) was performed in an attempt to describe the characteristics that separate individuals into the two clusters in the principal coor-

dinate analysis (Q matrix). Unfortunately, the component analysis (available from the author) did not greatly simplify the data space. The first eight principal component axes (64% of the variance) were studied. Only one cluster was produced in the analysis, a result that is consistent with hybridization but does not characterize individuals in the two principal coordinate clusters.

Means and standard errors. The means and their standard errors of each of the 35 characters for individuals classified as *C. californicum, C. occidentale,* and for individuals of each of the principal coordinate clusters were examined next for differences and relations in characteristics between members of the two coordinate clusters (Table 1). In the positive cluster, 34% of the character means are not significantly different from *C. occidentale* (within 2 standard errors) but are significantly different from *C. californicum,* and 26 percent do not differ from *C. californicum* but do differ from *C. occidentale.* The negative cluster has 31% of the character means non-differentiable statistically from *C. occidentale* but different from *C. californicum,* and 23% not different from *C. californicum* but different from *C. occidentale.* Forty-three percent of the characters have means significantly different between the plus and minus clusters. Individuals of the plus cluster have arachnoid pubescence on both peduncle and stem leaves, have long peduncle leaves, and wider inflorescence bracts, as does *C. californicum,* while having the number of leaf lobes, spine lengths on leaves, stem diameter, pappus length, and corolla lobe shape similar to *C. occidentale.* The negative cluster has these characters similar to the opposite parent, though not always as extreme. The minus cluster also has involucre shape like *C. occidentale,* but the plus cluster is quite variable in this character. Thus, the principal coordinate clusters are hybrid individuals composed of recombinant characteristics.

Silhouettes. Gestalt description of morphological variation of *Cirsium* in Happy Canyon is supplied by the silhouettes of stem leaves, inflorescence, and peduncle leaves (Figs. 6, 7, and 8). Recombinant types exist in the leaf silhouettes (Fig. 6). Again, a complete gradation of forms is seen in capitula (Fig. 7), and in the peduncle leaves (Fig. 8).

In the morphological analysis of the Happy Canyon population, organ silhouettes and a wide variety of numerical methods all suggest that, although the population includes two recognized taxonomic species, it is composed primarily of hybrids and backcross forms.

Pollen fertility. Sixty-seven percent of the individuals sampled had pollen fertility higher than 90%, and 85% of the population had pollen fertility higher than 80%. Unless the individuals with low fertility are all F_1 or some other specific type hybrid, no evidence emerged to indicate a sterility barrier. Apparently only one genetic population exists.

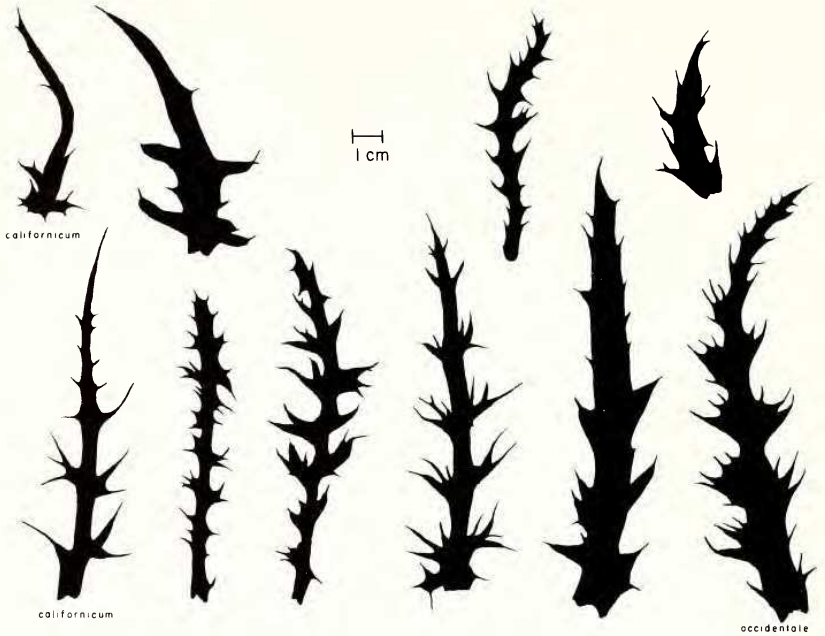

FIG. 6. Stem leaf silhouettes. Typical *C. occidentale* leaf is depicted lower right, and typical *C. californicum* leaf upper and lower left. A gradation of forms existed from one species to the other species.

A distance diagram (Fig. 9) may reveal a clustering of infertile hybrid individuals. If found, such clustering would suggest that two biological species, rather than one, were being studied. Individuals with low fertility were uniformly distributed across the morphological spectrum. Thus, pollen sterility does not restrict gene migration and recombination in the *Cirsium* population studied.

Ovule development. Another test for sterility barriers was that of ovule development per head in experimental crosses (Table 2). Those capitula that were bagged rarely produced any developed ovules. All cross-pollinated inflorescences produced a markedly greater proportion of developed ovules than did bagged plants. There are two hypotheses about out-crossing ovule development that should be tested. The first is that one or more types of crosses between different individuals (*occidentale* × *occidentale, californicum* × *californicum, occidentale* × *californicum, occidentale* × hybrid, *californicum* × hybrid, or hybrid × hybrid) will have significantly higher or lower percent ovule development than the other crosses. The other hypothesis is that crosses between individuals of the same species (*occidentale* × *occidentale,* and *californicum* × *californicum*) will have significantly higher or lower

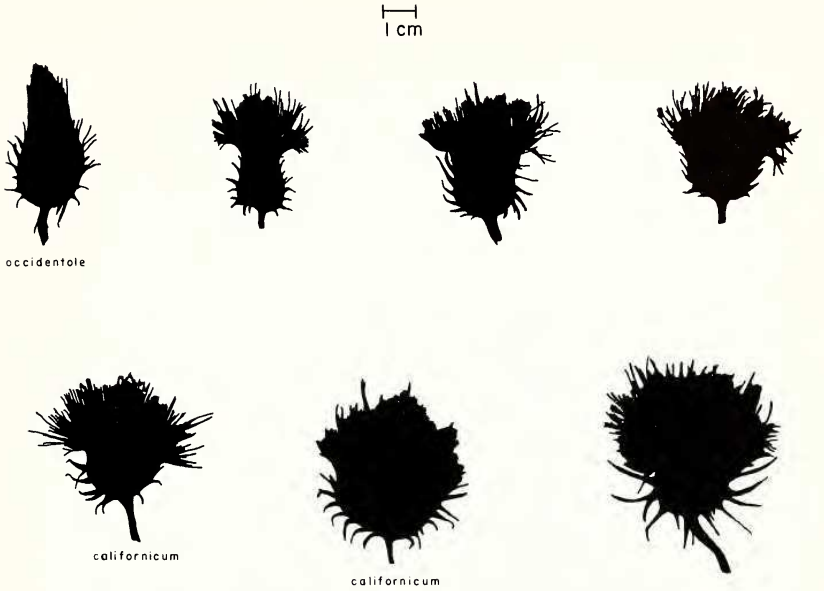

FIG. 7. Inflorescence silhouettes. Typical *C. occidentale* form is shown by the upper left inflorescence, and typical *C. californicum* forms by the lower left and center silhouettes. A gradation of forms existed from one species to the other.

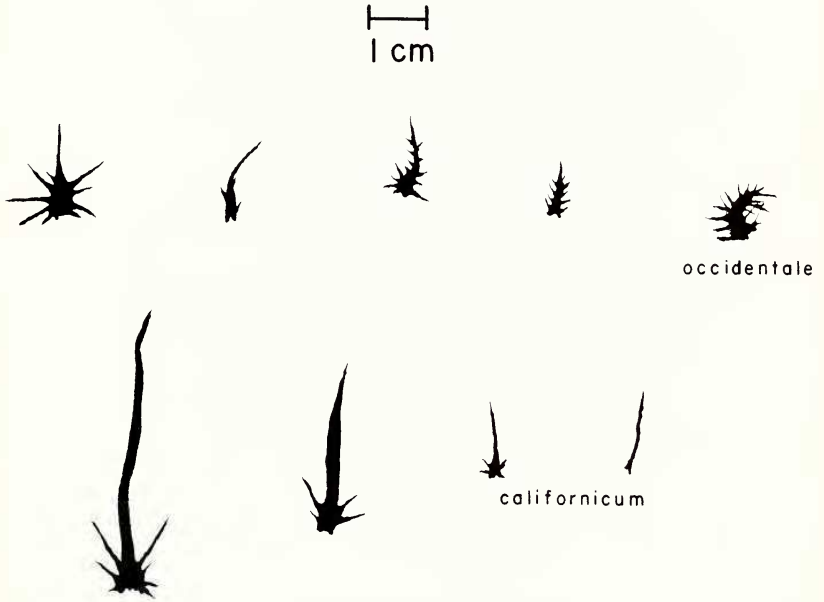

FIG. 8. Peduncle leaf silhouettes. Typical *C. occidentale* form is depicted by the upper right silhouette and typical *C. californicum* forms by the lower right two leaves. A wide variety of recombinant forms existed, a few of which are shown.

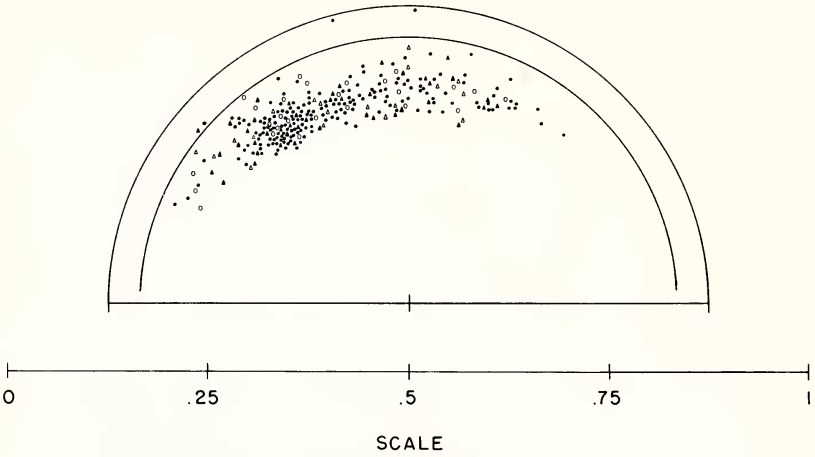

FIG. 9. Distance diagram of reference and Happy Canyon individuals. Symbols refer to percent pollen fertility of the individuals. Sterility was randomly distributed throughout the scatter. Thus, there was no evidence of a sterility barrier to gene recombination. ● = pollen fertility >95%, ▲ = pollen fertility between 90% and 95%, ○ = pollen fertility 80% to 90%, and △ = pollen fertility below 80%.

percent ovule development than crosses between species, species × hybrid, or hybrid × hybrid. These two hypotheses were tested by use of a one-way analysis of variance on the arcsine transformed data (arcsine $\sqrt{\text{frequency}}$ developed ovules per head). Neither test showed a significant difference (Table 2), so the null hypothesis that all types of crosses are equally fertile cannot be rejected.

Finally, developed ovule frequency in capitula of controlled lath house crosses was compared to the frequency of developed ovules per head in the field. In nature, mean percent developed ovules was 20.1 (S.D. = 25.9 n = 1160 flowers dissected). The non-developed ovaries were small and shriveled as if fertilization had not occurred. It may be that the efficiency of the pollen vectors in transporting pollen limits ovule development, as shown by Levin (1968) for *Lithospermum*. Developed ovules of capitula in experimental crosses were also about 20%.

The principal coordinate analysis separated *Cirsium* individuals into two distinct groups based on morphological characters. These clusters do not correspond to the taxonomic species. Could different environments have caused the observed phenotypic variation? This possibility was examined by asking whether a correlation exists between the defined habitat types and morphological phenotypes (Table 3). The null hypothesis is that the number of individuals in a cell is dependent only on the frequency of individuals sampled from a habitat and that the distribution of phenotypes is identical in each habitat. The null hy-

TABLE 2. OVULE DEVELOPMENT FREQUENCY IN EXPERIMENTAL CROSSES.

Bagged plants: $\bar{x} = 0.00097$; n = 18. (Mean frequency)
Cross pollinated: Frequency developed ovules, $\bar{x} = 0.2117$; n = 18

	Cross (group)					
Cross #	1 *californicum californicum*	2 hybrid hybrid	3 *occidentale occidentale*	4 *californicum* Hybrid	5 *occidentale* Hybrid	6 *californicum occidentale*
1	0.1875	0.1739	0.1250	0.2127	0.5650	0.1429
2	0.2698	0.1633	0.2500	0.2000	0.2222	0.1020
3	0.2500	0.2413	0.1515	0.1875	0.1892	0.1765

Analysis of variance on: Arcsine transformed $\sqrt{\text{frequency}}$ developed ovules.

	Anova				
Source of variation	df	SS	MS	F	
Among groups	5	267.96	53.59	1.52	Not significant
Within groups	12	422.89	35.24		
Total	17	690.85			
Groups 1 + 3 vs. 2 + 4 + 5 + 6	1	0.639	0.639	0.018	Not significant

TABLE 3. RELATION BETWEEN OCCURRENCE OF INDIVIDUALS IN THE CLUSTER DEFINED BY THE 3RD PRINCIPAL COORDINATE ANALYSIS AXIS AND HABITAT TYPE.

Principal coordinate axis III	Habitat			Total
	Grassland	Oak forest	Chaparral	
+ Cluster	15	3	31	49
− Cluster	93	51	39	183
Total	108	54	70	232

$\chi^2 = 33.8$ df = 2; $p \ll 0.001$; G.E.T. $p = 5.7 \times 10^{-8}$.

TABLE 4. RELATION BETWEEN OCCURRENCE OF INDIVIDUALS OF ELECTROPHORESIS LEUCINE AMINOPEPTIDASE PHENOTYPES AND HABITAT TYPE.

	LAP phenotype		
Habitat	Fast band only	Fast and slow bands	Total
Oak forest	11	27	38
Grassland	35	114	149
Chaparral	46	54	100
Total	92	195	287

$\chi^2 = 14.1$ df = 2; $p < 0.001$; G.E.T. $p = 0.000757$.

pothesis must be rejected (G.E.T. $p = 5.7 \cdot 10^{-8}$; $\chi^2 = 33.8$, df = 2, $p < 0.001$) based on the data of Table 3. Thus, it appears that habitat type is associated with phenotype.

Individual genetic traits were examined by isozyme analysis in 287 offspring rosettes via slab acrylamide gel electrophoresis. The malate dehydrogenase, lactate dehydrogenase, and "general protein" stains were monomorphic and will not be discussed further.

The leucine aminopeptidase (LAP) results are presented in Table 4, which relates the results to habitat type. The differences are denoted slow and fast, where slow bands remained closer to the origin than the fast bands. LAP electrophoretic phenotypes all contained the fast band, but some contained a slow band while others did not. Both the chi-square and generalized exact test were performed (G.E.T. $p = 0.000757$; $\chi^2 = 14.1$, df = 2, $p < 0.001$). Thus, LAP genotype distribution was correlated to habitat type, which suggests that morphs correlated to habitat may also have a genetically based component. The alkaline phosphatase and acid phosphatase data are published elsewhere (Wells and King 1980). The alkaline phosphatase genotypes resembled those of LAP in being correlated with habitat type (G.E.T. $p = 0.023$) whereas those of acid phosphatase were uniformly distributed (G.E.T. $p = 0.412$).

The correlation between habitat and both morphological and electrophoretic phenotypes presents an additional question. Is micro-habitat selection actually occurring, rather than an elevationally related cline, or even selection by some environmental factor(s) not determining habitat?

Hypotheses generated by these questions can be tested because of the population structure of *Cirsium* in Happy Canyon, i.e., the spatial and elevational isolation of grassland region 3 from 1 & 2. Morphologically, 9.1% of the *Cirsium* of grassland No. 3 are from the + cluster (n = 11), in comparison to 14.4% of grassland *Cirsium* 1 & 2 (n = 97). LAP electrophoretic data had 13.0% of grassland region 3 *Cirsium* with only the fast band (n = 23), while 25.4% of area No. 1 & 2 *Cirsium* had only the fast band (n = 126).

If a cline or a nonhabitat-related environmental factor were operative, then, grassland *Cirsium* colonies 1 & 2 should differ from No. 3, and both grassland *Cirsium* No. 3 and No. 1 & 2 should differ from the intervening chaparral *Cirsium*. However, if habitat-related selection is occurring then one would expect *Cirsium* in grassland habitats 1 & 2 and 3 not to be significantly different, but that they would differ from those *Cirsium* found in the chaparral.

Grassland *Cirsium* in regions 1 & 2, as defined morphologically by the principal coordinate analysis, did not differ significantly from those in region 3 (G.E.T. $p = 0.72$, $\chi^2 = 0.21$, df = 1, $p > 0.6$), but each did differ significantly from the chaparral *Cirsium* (grassland 1 & 2 vs. chaparral: G.E.T. $p = 2.19 \times 10^{-5}$; $\chi^2 = 18.29$, df = 1, $p <$

0.005; grassland 3 vs. chaparral: G.E.T. $p = 0.0431$; $\chi^2 = 4.93$, df = 1, $p < 0.05$). The LAP isozyme data showed the same pattern of differences as morphology (grassland 1 & 2 vs. 3: $\chi^2 = 1.65$, df = 1, $p > 0.2$; grassland 1 & 2 vs. chaparral: $\chi^2 = 10.50$, df = 1, $p < 0.005$; grassland 3 vs. chaparral: $\chi^2 = 8.47$, df = 1, $p < 0.005$). Alkaline phosphatase data (Wells and King 1980), however, showed significant differences only between grassland 1 & 2 and chaparral *Cirsium* (G.E.T. $p = 0.0265$), but not between the grassland 1 & 2 and 3 *Cirsium* (G.E.T. $p = 0.84$), or between grassland 3 and chaparral *Cirsium* (G.E.T. $p = 0.0682$).

Conclusions

1. The Happy Canyon *Cirsium* population consists of one biological species with no sterility barriers.
2. Morphological and electrophoretic phenotypes correlated with habitat type appear to have a genetic basis.
3. The differentiation corresponding to habitat types suggests that several phenotypic traits may be subject to selection and that differentiation along new lines may have resulted after hybridization of *C. californicum* and *C. occidentale* in the Happy Canyon population.

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